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## Modeling of 1,2-Dichloroethane Biodegradation by *Xanthobacter autotrophicus* GJ10 under Shock Loading of Other Halogenated Compounds in Continuous Stirred Tank Bioreactor

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A mathematical model describing the behavior of a continuous culture that degrades 1,2-dichloroethane and receives a shock loading of another compound was developed. The model takes into account possible cell death due to toxicity, growth inhibition and additional growth of cells on the second carbon source. Biodegradation is coupled to cell growth on the additional carbon source or by incomplete and unproductive degradation.

The model was tested with *Xanthobacter autotrophicus* strain GJ10 growing on 1,2-dichloroethane in a continuous stirred tank bioreactor. Dichloromethane, dibromomethane, 1,2-dibromoethane, monofluoroacetate, monochloroacetate and monochloroacetic acid were added separately in the form of a pulse. The effects that were observed varied from low toxicity in case of dihalomethanes and chloroacetate up to severe cell death followed by culture washout in the case of monofluoroacetate and 1,2-dibromoethane. The experimental profiles were in most cases satisfactorily described with the proposed model.

*Key words:*

Bioreactor, biodegradation, halogenated compounds, mathematical modeling, toxicity, *Xanthobacter autotrophicus*

### Introduction

Large quantities of 1,2-dichloroethane (DCA), exceeding tens of millions of tons, are produced annually,<sup>1</sup> mainly for the synthesis of vinyl chloride. Improper disposal and leaks have led to numerous cases of soil and groundwater pollution. Because of its toxicity and carcinogenicity a vast amount of work has focused on its removal from polluted water. Due to the low sorption coefficient of DCA,<sup>2</sup> its removal by absorption on activated carbon is inefficient. As an alternative method, biodegradation by selected microbial strains has been proposed.<sup>3</sup> *Xanthobacter autotrophicus* strain GJ10 is the best studied aerobic degrader of DCA.<sup>3</sup> Several types of bioreactors containing this strain were tested in terms of removal efficiency and culture stability.<sup>4</sup> Successful attempts were made to obtain mixed cultures of GJ10 and another strain that are suitable for the degradation of DCA and vinyl chloride<sup>5</sup> or DCA and monochlorobenzene.<sup>6</sup> Much less work has been aimed at establishing the stability of strain GJ10 upon shock feeding. This problem appears to be important in practice since industrial effluents usually

contain a mixture of pollutants of variable concentrations.<sup>7</sup> Moreover, some of such pollutants may appear only temporarily and in high concentration, exerting a strong effect on the microbial culture.

The adverse effect of xenobiotics on bacterial cells may have different causes, such as energy uncoupling caused by membrane damage due to the toxic compound itself,<sup>8,9</sup> inactivation of essential enzymes caused by mechanism-based inactivation of enzymes, formation of reactive intermediates that act as alkylating agents, and formation of electrophilic alkylating compounds during biotransformation.<sup>10,11</sup> The effects exerted by xenobiotics in continuous culture are more difficult to describe than in batch culture. This is due to changes in concentrations of biomass and compounds that will occur as a result of washout and air stripping, which especially holds for systems which are not in steady state due to perturbations such as shock loadings.

The work presented here aims to model the behavior, in terms of compound removal, of a continuous culture that utilizes a chlorinated hydrocarbon and receives shock loadings of second halogenated compound. By means of the developed mathematical model one can analyze in general the nature of the adverse effects exerted of the added compound

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to the culture: toxicity due to reasons mentioned above,<sup>8–11</sup> growth inhibition because of relatively high initial concentrations of the main substrate or the pulsed compound, as well as the possible use of the added compound as a carbon source for additional microbial growth and the secondary transformation of the pulsed compound by the cells. The possibilities of the model are illustrated on five short-chain halogenated compounds added separately in the form of a pulse to a stirred bioreactor containing *X. autotrophicus* GJ10 that degrades and utilizes 1,2-dichloroethane. The compounds were selected on a basis of previous research<sup>12,13</sup> that revealed their different effects on strain GJ10 in a batch culture. They are: dichloromethane (DCM) and dibromomethane (DBM) known as slight inhibitors and almost inert for the strain; dibromoethane, known as a very strong toxin and inhibitor of the strain growth; monofluoroacetic acid (as acetate) known as not convertible by this strain and toxic one; and monochloroacetic acid (as free acid and as acetate) being inhibitor but a carbon source for growth as well.<sup>13</sup>

## Materials and methods

### Materials

*Xanthobacter autotrophicus* strain GJ10<sup>3</sup> was used in all experiments. The strain was grown on MMY medium containing (g L<sup>-1</sup>): 0.46 Na<sub>2</sub>HPO<sub>4</sub> · 12H<sub>2</sub>O, 0.16 KH<sub>2</sub>PO<sub>4</sub>, 0.5 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2 MgSO<sub>4</sub> · 7H<sub>2</sub>O, 5 mL L<sup>-1</sup> trace metal solution<sup>12</sup> and 30 mg L<sup>-1</sup> yeast extract. The main substrate (DCA) was dissolved in the growth medium. All chemicals used were of > 98 % purity except for sodium fluoroacetate, which was of 95 % purity. All compounds were used as received.

### Methods

The experiments were carried out in a 3-liter continuous stirred tank bioreactor.<sup>11</sup> Operational conditions were as follows: liquid volume 2.5 L, aeration flow rate  $Q = 0\text{--}100$  mL min<sup>-1</sup>, stirring rate 400 rpm, dilution rate of the liquid phase  $0.025\text{--}0.027$  h<sup>-1</sup>, temperature  $30 \pm 0.1$  °C, pH  $7.0 \pm 0.1$ . The substrate ( $15\text{--}20$  mmol L<sup>-1</sup> DCA) was continuously fed to the fermentor together with the growth medium through Viton rubber tubing. DCM, DBM, DBE, MFA and MCA were added with a sterile syringe (each in a separate experiment) directly to the fermentor. Samples were regularly taken from the inlet and from the outlet of the CSTB. The response of the bioreactor to the pulses was followed by measuring the concentrations of biomass, both substrates (DCA and the compound added) and degradation products.

### Analyses

All haloalkanes were analyzed by gas chromatography. Aqueous samples of 1 or 2 mL (depending on the expected concentration range) from the fermentor, containing haloalkanes were extracted with 1 mL of diethyl ether containing 0.1 mmol L<sup>-1</sup> 1-chlorohexane as an internal standard. Samples of 5 µL were injected manually with a Hamilton syringe in a Chrompack 9001 gas chromatograph equipped with a CP-Sil-5CB column and a FID detector. The oven was temperature programmed as follows:  $t = 3$  min isothermal at 30 °C followed by an increase of temperature change  $10$  °C min<sup>-1</sup> to 150 °C. DCM was analyzed separately with a model 6890 gas chromatograph (Hewlett-Packard) equipped with an HP-5 column, autosampler and electron capture detector. The temperature program was as follows: 5 min isothermal at 30 °C followed by an increase of temperature change  $10$  °C min<sup>-1</sup> to 120 °C.

The concentrations of chloride and bromide, as well as fluoroacetate were analyzed by ion chromatography. A Dionex type DX-120 chromatograph equipped with an Alltech column type Allsep A-2 was used. The eluent was prepared by dissolving  $2.7$  mmol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> and  $0.3$  mmol L<sup>-1</sup> NaHCO<sub>3</sub> in deionized water.

The other haloacetates and glycolate were determined by HPLC using a Varian ChromSep SS Microspher C18 column and a UV detector working at 210 nm. The eluent was prepared as follows: deionized water and acetonitrile were mixed at a ratio of 7 : 3 and  $680$  mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> and  $340$  mg L<sup>-1</sup> of sodium dodecylsulphate were added. The pH was adjusted with H<sub>3</sub>PO<sub>4</sub> to a value of 3. Finally, the solution was mixed 1 : 1 with deionized water and used as eluent. Concentrations of organic compounds were calculated from calibration curves for all of the chromatographic analyses.

The biomass concentrations were measured as optical density at  $\lambda = 500$  nm and calculated from a calibration curve.

### Mathematical model

The following mass balance is usually applied for the description of a CSTB with one growth-limiting substrate:<sup>8</sup>

$$\frac{dy_X}{dt} = -D \cdot \gamma_X + \mu \cdot \gamma_X \quad (1)$$

$$\frac{dy_S}{dt} = D \cdot (\gamma_{S_{inlet}} - \gamma_S) - \frac{1}{Y_{X/S}} \cdot \mu \cdot \gamma_X \quad (2)$$

As mentioned in the Introduction, the addition of a second substrate to a CSTB can exert different effects on the microbial culture. For the purpose of

modeling of the CSTB for use in DCA degradation we assumed that one or more of the following effects can occur upon addition of second compound:

a) toxicity resulting in biomass decay. The rate of cell death was assumed to be proportional to culture density and toxicant concentration.<sup>14,15</sup> For the surviving cells we have:

$$r_X = \frac{d\gamma_X}{dt} = \mu \cdot \gamma_X - \alpha \gamma_I \gamma_X \quad (3)$$

or

$$\gamma_X = \gamma_{X,0} \exp(\mu t) \exp(\alpha \gamma_I t)$$

The coefficient  $\alpha$  can be related to the so-called median lethal concentration ( $LC_{50}$ ) of the compound I, which is the concentration at which 50 percent of the organisms die after a certain exposure time during the toxicity test.<sup>14,16</sup> Under these definitions the  $LC_{50}$  will be the concentration  $\gamma_{I,1/2}$ , evaluated from eq. (3), provided the microbial culture is in the stationary phase in a batch culture, i.e. when  $\mu \approx 0$ .

$$\ln \frac{0.5\gamma_{X,0}}{\gamma_{X,0}} = -\ln 2 = -\alpha \gamma_{I,1/2} t_{1/2}$$

b) inhibition of cell growth, caused by the added compound and by DCA. The latter was described by Ferreira Jorge and Livingston<sup>17</sup> who adopted the Luong model of inhibition.<sup>18</sup> To describe the inhibition by the pulsed compound we adopted the equation of Yersulimsky,<sup>19</sup> which contains an inhibition constant ( $K_i$ ) that can be found by fitting. Thus, the cell growth rate on the main substrate (DCA) can be given by the following equation, combining Luong's and Yersulimskii's models:

$$\mu_S = \mu_{\max,S} \cdot \frac{\gamma_S}{K_S + \gamma_S} \cdot \frac{\gamma_{S,m} - \gamma_S}{\gamma_{S,m}} \cdot \frac{K_i}{K_i + \gamma_I}, \quad \gamma_S < \gamma_{S,m} \quad (4)$$

$$\mu_S = 0, \quad \gamma_S \geq \gamma_{S,m}.$$

In this equation  $K_i$  is inhibition constant. The latter parameter  $\gamma_{S,m}$  implies that no growth is possible at a main substrate (DCA) concentration equal to or exceeding the threshold value  $\gamma_{S,m}$ . Eq. (4) accounts for inhibition effects on microbial growth caused by the added compound, besides the toxicity, expressed by  $\alpha$ .

c) additional growth of biomass due to the presence of another carbon substrate.

All of these effects are modeled by introducing the growth rate  $\mu_I$  for the added compound as a substrate with the added compound as inhibitor:

$$\mu_I = \mu_{\max,I} \cdot \frac{\gamma_I}{K_{S,I} + \gamma_I} \cdot \frac{\gamma_{S,m} - \gamma_S}{\gamma_{S,m}}, \quad \gamma_S < \gamma_{S,m};$$

$$\mu_I = 0, \quad \gamma_S \geq \gamma_{S,m}.$$

Note that the Luong factor  $(\gamma_{S,m} - \gamma_S)/\gamma_{S,m}$  prevents growth on the second substrate I when  $\gamma_S \geq \gamma_{S,m}$ .

The secondary compounds that are added in the form of a pulse can be removed in three ways: by washout with the aqueous phase, by stripping via the gas phase, or by biological activity. The last phenomenon can be associated with biomass growth provided that the products of the conversion of the substrate are further used by cells. In this case the biological degradation rate is given by:

$$r_{I,growth} = -\frac{\mu_I}{Y_{X/I}} \cdot \gamma_X \quad (6)$$

If the products of the conversion of the second compound are not utilized for growth, for example as in case of a pulse of DBE,<sup>12</sup> then  $\mu_{\max,I} = 0$  and the rate of biological removal of the compound can be described with the following first-order kinetic equation:

$$r_{I,non-growth} = -\beta \cdot \gamma_X \cdot \gamma_I \quad (7)$$

Taking into account the equations (1 – 7) the following mass balance for the considered two-phase CSTB can be formulated:

$$\frac{d\gamma_X}{dt} = (\mu_S + \mu_I) \cdot \gamma_X - \alpha \cdot \gamma_I \cdot \gamma_X - D \cdot \gamma_X$$

$$\frac{d\gamma_S}{dt} = D \cdot (\gamma_{S,inlet} - \gamma_S) - \frac{\mu_S}{Y_{X/S}} \gamma_X - k_L a \left( \gamma_S - \frac{\gamma_{S,g}}{H_S} \right)$$

$$\frac{d\gamma_{S,g}}{dt} = k_L a \cdot \left( \gamma_S - \frac{\gamma_{S,g}}{H_S} \right) \frac{V_L}{V_g} - D_g \gamma_{S,g} \quad (8)$$

$$\frac{d\gamma_I}{dt} = -D \cdot \gamma_I - \frac{\mu_I}{Y_{X/I}} \gamma_X - k \cdot \beta \cdot \gamma_X \cdot \gamma_I - j \cdot k_L a \left( \gamma_I - \frac{\gamma_{I,g}}{H_I} \right)$$

$$\frac{d\gamma_{I,g}}{dt} = j \cdot k_L a \left( \gamma_I - \frac{\gamma_{I,g}}{H_I} \right) - j \cdot D_g \gamma_{I,g}$$

$$\frac{d\gamma_P}{dt} = -D \cdot \gamma_P + 2 \frac{M_{Cl}}{M_S} \cdot \frac{\mu_S}{Y_{X/S}} \cdot \gamma_X + i \frac{M_{Cl}}{M_I} \gamma_X \left( \frac{\mu_I}{Y_{X/I}} + k \cdot \beta \cdot \gamma_I \right)$$

The initial conditions ( $t = 0$ ) are:

$$\gamma_X = \gamma_{X,0}, \quad \gamma_S = \gamma_{S,0}, \quad \gamma_P = \gamma_{P,0},$$

$$\gamma_I = \gamma_{I,0}, \quad \gamma_{S,g} = \gamma_{S,g,0}, \quad \gamma_{I,g} = 0$$

The model variables from eqs. (3–8) and their preset values are presented in Table 1. The parameters  $K_{S,I}$  for the dihalomethanes were estimated as the other unknown parameters starting from different initial approximations. It was also assumed for DBE and MFA that  $\mu_{\max,I}$  is equal to zero since no growth was observed on these compounds in batch cultures.<sup>13</sup>

For the purpose of estimation of the mass transfer coefficient  $k_L a$ , the general correlation<sup>23</sup>

$$k_L a_1 \cdot Q_{g,1}^{0.4} = k_L a_2 \cdot Q_{g,2}^{0.4} \quad (9)$$

was used. The reference value of  $k_L a_1$  (equal to 0.5 h<sup>-1</sup>) was estimated for  $Q_{g,1} = 6 \text{ L h}^{-1}$  for oxygen by the dynamic method using the time variation of dissolved oxygen concentration in the broth after sudden stop and further resuming the aeration.<sup>24</sup> The formula (9) was used for re-calculation of  $k_L a$  for each experiment. The factors  $j$  and  $k$  were equal to 0 or 1 and the factor  $i$  was equal to 0, 1 or 2 depending on the chlorine content in the compound added to the CSTB.

## Experimental results

During all of experiments the steady state and the practically complete degradation of DCA prior to the shock loading was observed. The outlet biomass concentration (calculated from the optical density of the culture) remained constant too. It dif-

fered for the different pulsed compounds within 0.235 and 0.322 kg m<sup>-3</sup>.

Different effects were observed after shock loadings of five xenobiotics to the continuous culture of the strain GJ10 (Fig. 1–6).

DCM (Fig. 1) was poorly degraded by the cells as indicated by the modest increase in the chloride concentration in the medium. DCM was mostly removed in an abiotic way due to its high volatility.

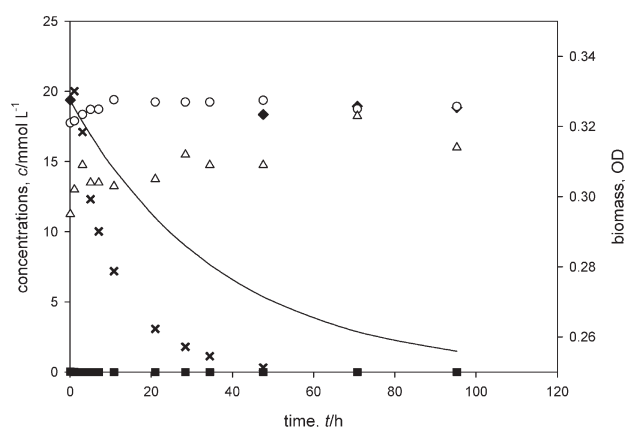


Fig. 1 – Experimental profiles of total biomass, DCA, DCM and chloride for the pulse of DCM in CSTB. Symbols: (Δ) – biomass (OD, right-hand side ordinate), (○) – chloride, (×) – DCM; (◆) – DCA, inlet; (■) – DCA – outlet. Solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

DBM (Fig. 2) exhibited a mild toxic effect and it was dehalogenated by the cells more rapidly than DCM. On the other hand, DBM could be partially utilized as a co-substrate and supported the strain growth. The calculations showed that the sum of DBM and bromide molar concentrations satisfied the initial DBM concentration until the 6<sup>th</sup> hour af-

Table 1 – Model parameters related to the tested substances: as a main substrate (S) or added compound (I)

Compound, type	$H, (-)$	$K_S$ kg m <sup>-3</sup>	$\mu_{\max}$ h <sup>-1</sup>	$Y_{X/S}/\text{kg}_{\text{biomass}}$ kg <sup>-1</sup> <sub>substrate</sub>	$\alpha$ m <sup>3</sup> kg <sup>-1</sup> h <sup>-1</sup>	$\beta$ , m <sup>3</sup> kg <sup>-1</sup> h <sup>-1</sup>	$K_i$ kg m <sup>-3</sup>	$i$	$j$	$k$
DCA (S)	0.058 <sup>1</sup>	0.026	0.104	0.23 <sup>2</sup>	0	0	$\infty$	2	1	0
DCM (I)	0.110 <sup>1</sup>	To be evaluated	To be evaluated	To be evaluated	To be evaluated	0	To be evaluated	2	1	0
DBM (I)	0.057 <sup>3</sup>	To be evaluated	To be evaluated	To be evaluated	To be evaluated	0	To be evaluated	0	1	0
DBE (I)	0.034 <sup>4</sup>	Not relevant	0	0	To be evaluated	To be evaluated	To be evaluated	0	1	1
MFA (I)	Non-volatile	Not relevant	0	0	To be evaluated	0	To be evaluated	0	0	1
MCA (acid, I)	Non-volatile	0.0123 <sup>5</sup>	To be evaluated	0.155 <sup>5</sup>	To be evaluated	To be evaluated	To be evaluated	1	0	1
MCA (salt, I)	Non-volatile	0.0123 <sup>5</sup>	To be evaluated	0.155 <sup>5</sup>	To be evaluated	To be evaluated	To be evaluated	1	0	1

<sup>1</sup>See ref. 20

<sup>2</sup>See ref. 17

<sup>3</sup>See ref. 21

<sup>4</sup>See ref. 22

<sup>5</sup>See ref. 13



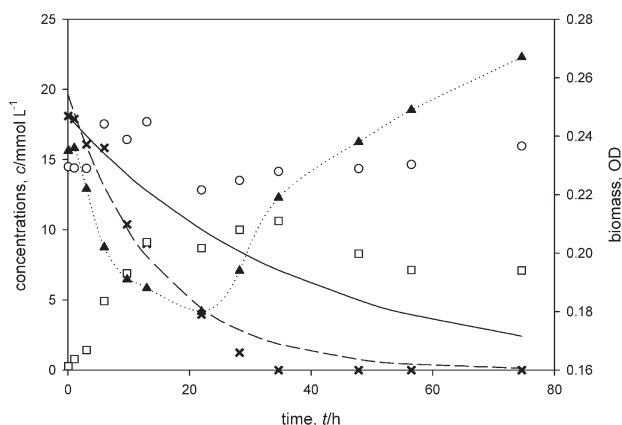


Fig. 2 – Experimental profiles of total biomass, DCA, DBM, chloride and bromide for the pulse of DBM in CSTB. Symbols: ( $\blacktriangle$ ), dotted line – biomass (OD, right-hand side ordinate); ( $\times$ ), dashed line – DBM; ( $\circ$ ) – chloride; ( $\square$ ) – bromide; solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

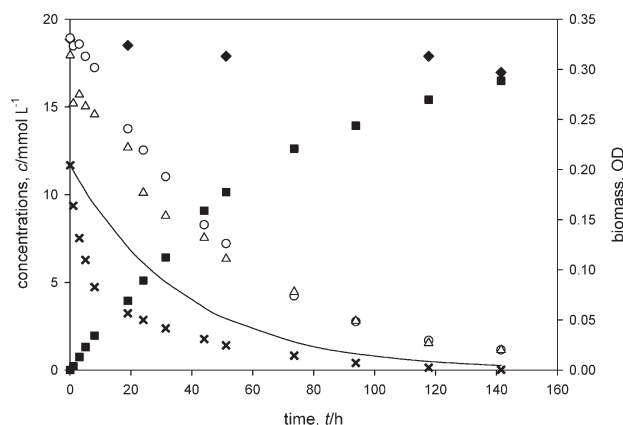
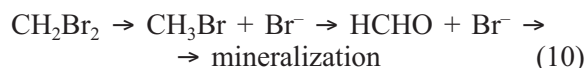


Fig. 3a – Experimental profiles of biomass and the chlorine balance for the pulse of DBE into the CSTB. Symbols: ( $\Delta$ ) – biomass (OD, right-hand side ordinate), ( $\circ$ ) – chloride, ( $\times$ ) – DBE; ( $\blacklozenge$ ) – DCA, inlet; ( $\blacksquare$ ) – DCA, outlet. Solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

ter injection. It means that DBM degradation and the associated bromide release went much faster than wash-out by the feed solution at the set dilution rate ( $0.027 \text{ h}^{-1}$ ). The bromide concentration remained considerable even after DBM was washed out. This slower decrease of bromide concentration even after total DBM degradation and wash-out could be explained by the accumulation of an intermediate product, e.g. monobromomethane, to yield bromide with a further formation of formaldehyde and mineralization:<sup>12</sup>



Fast biomass recovery after its temporary decay was observed when DBM was removed.

The next substrate tested, DBE (Fig. 3a, b), caused almost complete washout of the culture within 140 h. The compound is a good substrate for enzymes of strain GJ10 as indicated by the fast decrease of biomass concentration in the first 10 h, accompanied by bromide production. After 10 h the production of bromide stopped and accumulation of an intermediate, monobromoacetate, occurred (Fig. 3b). Comparison of the removal rates calculated from the experimental data for DBE ( $0.045 \text{ h}^{-1}$ ) and MBA ( $0.035 \text{ h}^{-1}$ ), with the liquid phase dilution rate ( $0.027 \text{ h}^{-1}$ ) confirms that both DBE and MBA were partially converted by the biomass. In this experiment the chloride concentration in the reactor decreased, indicating that liberation of chloride from DCA was strongly retarded after addition of DBE. The sum of the outlet molar concentrations of chloride and DCA practically satisfied the mass balance (Fig. 3a).

When MFA was added to the bioreactor (Fig. 4), a complete washout of the culture was observed, similar to what was found with DBE. However, un-

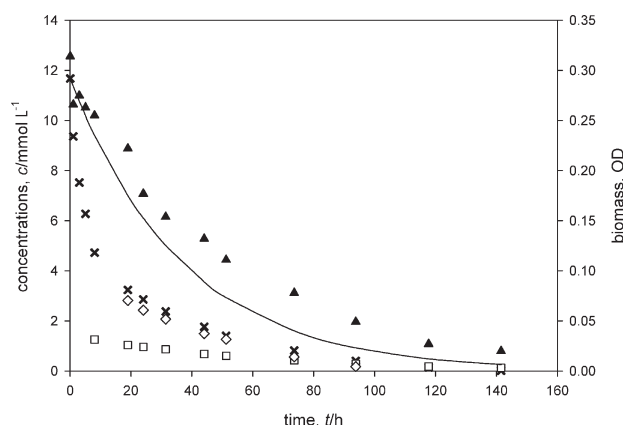


Fig. 3b – Experimental profiles of biomass and the bromine balance for the pulse of DBE into the CSTB. Symbols: ( $\blacktriangle$ ) – biomass (OD, right-hand side ordinate), ( $\times$ ) – DBE; ( $\diamond$ ) – MBA. Solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

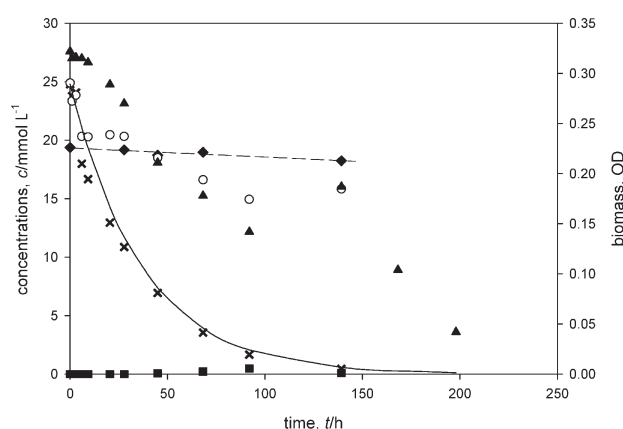


Fig. 4 – Experimental profiles of biomass, chloride and MFA for the pulse of MFA into CSTB. Symbols: ( $\blacktriangle$ ) – biomass (OD, right-hand side ordinate); ( $\circ$ ) – chloride, ( $\times$ ) – MFA; ( $\blacklozenge$ ) – DCA, inlet; ( $\blacksquare$ ) – DCA, outlet. Solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

like the situation with DBE, no conversion of MFA was observed. This conclusion was supported by the removal rate of MFA ( $0.028 \text{ h}^{-1}$ ) which was close to the dilution rate ( $0.027 \text{ h}^{-1}$ ). It means that MFA practically was not digested by the strain.

The last tested substrate, MCA, was introduced to the bioreactor as acid (Fig. 5) or as sodium monochloroacetate (Fig. 6). In the case of MCA as acid a temporary pH drop occurred due to its strong acidity ( $\text{pK}_a = 2.82$ ). After shock loading with MCA as acid the culture density decreased at a rate of  $0.024 \text{ h}^{-1}$ , close to the dilution rate  $0.025 \text{ h}^{-1}$ , i.e. there was practically no microbial growth, cf. Fig. (5). However, the MCA-acid was still degraded by the remaining biomass, causing the removal of MCA with a rate higher than the dilution rate. Under these conditions conversion of MCA to glycolic acid and further degradation of the latter took place. The calculated removal rate for glycolic acid ( $0.057 \text{ h}^{-1}$ ) was twice as high as the pre-set dilution rate. After MCA was removed from the bioreactor, cell growth was re-established with a specific growth rate  $\mu = 0.064 \text{ h}^{-1}$ , corresponding to almost complete DCA biodegradation through glycolate, (Fig. 5).

A different response of the culture was recorded when the MCA was neutralized with sodium hydroxide before its addition (Fig. 6). In this case biomass concentration changed only slightly and the removal of MCA followed by glycolate formation was more rapid than in the previous case. The latter was mineralized at a much higher rate ( $0.081 \text{ h}^{-1}$ ) than previously, obviously due to higher concentration of biomass.

## Modeling and discussion

The mathematical model presented by eqs. (3–8) contains 13 variables. Some of them could be separately evaluated or taken from the literature. The remaining 5 variables should be evaluated from the experimental data. In case of substrates that do not support growth (DBE, MFA, MCA-acid) or that do not cause chloride release (DBM, DBE, MFA) the number of the estimated parameter is from two to five, respectively. The model parameters are summarized in Table 1. One can see how many parameters have to be evaluated for each added compound.

The other variables associated with the experimental conditions are given in Table 2.

The experimental data were modeled using the equations for added compound, biomass, product and substrate described above (eqs. 3–8). The system of six differential equations (8) with initial conditions (9) was solved by the TUTSIM simulator<sup>25</sup> coupled with an optimization procedure according to

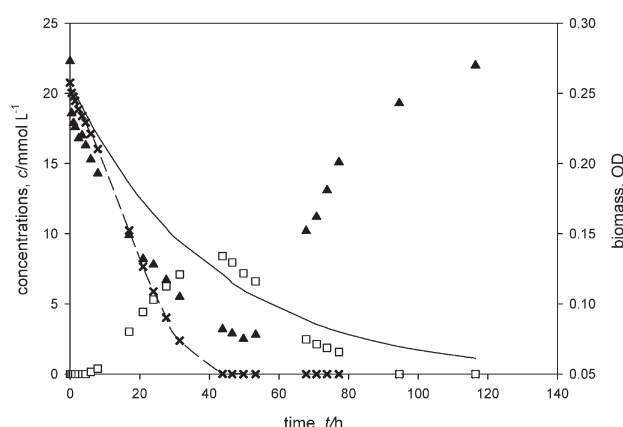


Fig. 5 – Experimental profiles of biomass, chloride, MCA-acid and glycolate (GLC) for the pulse of MCA-acid into CSTB. Symbols: ( $\blacktriangle$ ) – biomass (OD, right hand side ordinate), ( $\times$ ), dashed line – MCA, ( $\square$ ) – glycolate. Solid line – dilution curve,  $D_r = 0.025 \text{ h}^{-1}$ .

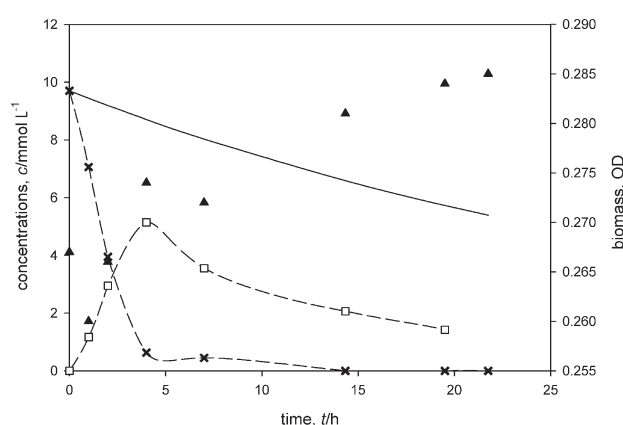


Fig. 6 – Experimental profiles of biomass, MCA and glycolate (GLC) for the pulse of MCA-acetate into CSTB. Symbols: ( $\blacktriangle$ ) – biomass, (OD, right-hand side ordinate); ( $\times$ ), – MCA-acetate, ( $\square$ ), dashed line – glycolate. Solid line – dilution curve,  $D_r = 0.027 \text{ h}^{-1}$ .

Table 2 – Experimental conditions

Parameter	Value	Source
$D, \text{ h}^{-1}$	0.025 – 0.029	Own experiments
$D_g, \text{ h}^{-1}$	0.01 – 20	Own experiments
$k_L a, \text{ h}^{-1}$	0.27 – 0.62	Own experiments, eq. (9)
$V_L/V_g$	5 – 9	Own experiments

the Nelder-Mead method. The function to be minimized was the sum of the squares of the differences between the experimental and the calculated values of the outlet concentrations of the product (chloride), the secondary substrate, and the total biomass  $\gamma_{X,\text{tot}}$  (dead and alive, read from the total determined optical density) for each moment of sampling:

$$\text{Sum} = \sum_i [(\gamma_{P,i}^{\text{calc}} - \gamma_{P,i}^{\text{exp}})^2 + (\gamma_{L,i}^{\text{calc}} - \gamma_{L,i}^{\text{exp}})^2 + (\gamma_{X,\text{tot},i}^{\text{calc}} - \gamma_{X,\text{tot},i}^{\text{exp}})^2]$$

and

$$\text{Sum} = \sum_i (\gamma_{S,i}^{\text{calc}} - \gamma_{S,i}^{\text{exp}})^2 + (\gamma_{P,i}^{\text{calc}} - \gamma_{P,i}^{\text{exp}})^2 + (\gamma_{I,i}^{\text{calc}} - \gamma_{I,i}^{\text{exp}})^2 + (\gamma_{X,tot,i}^{\text{calc}} - \gamma_{X,tot,i}^{\text{exp}})^2 \quad (11)$$

for the case of DBE.

The total biomass concentration  $\gamma_{X,tot}$  was calculated from the solution of the following differential equation, coupled with the system (8):

$$\frac{d\gamma_{X,tot}}{dt} = -D_r \gamma_{X,tot} + (\mu_S + \mu_I) \gamma_X \quad (12)$$

The procedure of optimization was started by giving the evaluated variables (cf. Table 1) different initial values and it was stopped when the sum of the squares reached a minimum value. Indications for the reliability and the physical sense of the estimated variables are the similar solutions and the similar values of the estimated parameters found when optimization was started with different initial values.

The parameter values estimated by the mathematical model from the experimental data for each added halogenated compound are given in Table 3.

Values for the toxicity parameter  $\alpha$  were greater than zero for all experiments indicating that all the compounds added in the form of a pulse exerted some toxicity to the biomass, even though most of them are also a carbon source for microbial growth.

The most toxic compound was DBE. In this case toxicity coefficient  $\alpha$  was  $403.8 \text{ M}^{-1} \text{ h}^{-1}$ . It is interesting to note, that considerable conversion of DBE in a non-growing culture was established, i.e. the rate constant  $\beta$ , accounting for this process is  $19.72 \text{ M}^{-1} \text{ h}^{-1}$ . DBE possesses strong toxicity and

significant inhibition effect on microbial growth ( $K_i = 0.505 \text{ kg m}^{-3}$ ). It does not support microbial growth, but it is biodegradable due to the remaining biomass. The effects of toxicity and inhibition can be explained by the high affinity of haloalkane dehalogenase for DBE, with a  $k_{\text{cat}} K_m^{-1}$  value for DBE that is two orders of magnitude higher than the respective value for DCA.<sup>26</sup> Furthermore, highly toxic products are formed when the DBE dehalogenation product 2-bromoethanol is oxidized to bromoacetaldehyde. The toxic effect of bromoacetaldehyde on the biomass is additionally modeled and discussed in the Appendix.

The parameters estimated for DBM lead us to the conclusion that it has very low toxicity ( $\alpha = 0.608 \text{ M}^{-1} \text{ h}^{-1}$ ), no inhibition and serve as secondary carbon source for additional, but slow microbial growth ( $\mu_{\text{max},I} = 0.024 \text{ h}^{-1}$ ,  $Y_{X/S} = 0.107$ ).

Comparison of the experimental data and the modeling curves for pulse of DBM and EDB is demonstrated in Figs. 7 and 8.

Dichloromethane (DCM) is also substrate for haloalkane dehalogenase but with much lower affinity than DCA<sup>25</sup> (higher value of  $K_s$  and lower yield coefficient,  $Y_{X/S} = 0.071$ ). It has low inhibition effect ( $K_i = 3.88 \text{ kg m}^{-3}$ ) and relatively strong toxicity ( $\alpha = 7.03 \text{ M}^{-1} \text{ h}^{-1}$ ) with slow microbial growth ( $\mu_{\text{max},I} = 0.017 \text{ h}^{-1}$ ).

MCA as acetate can serve as a sole carbon and energy source for strain GJ10 and it was rapidly dehalogenated and utilized as a carbon source after a shock loading. According to the values for the estimated variables mono-chloroacetate possesses low toxicity, no inhibition on microbial growth and considerable biodegradability with a high chloride yield.

Table 3 – Model parameters evaluated from experimental data by the mathematical model by optimization procedure

Compound, type (S, I):	$\mu_{\text{max}}$ $\text{h}^{-1}$	$K_{S,I}$ $\text{kg m}^{-3}$	$Y_{X/S}, Y_{X/I}$ $\text{kg}_{\text{biomass}} \text{ kg}_{\text{carbon source}}^{-1}$	$\alpha$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$	$\beta$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$	$K_i$ $\text{kg m}^{-3}$	$i$	$j$	$k$
DCA (S)	0.104*	0.026*	0.23*	0	0	$\infty$	2	1	0
DCM (I)	0.017	0.039	0.071	0.083	0	3.88	2	1	0
DBM (I)	0.021	0.052	0.107	0.0035	0	$\infty$	0	1	0
DBE (I)	0	Not relevant	0	2.15	0.105	0.505	0	1	1
MFA (I)	0	Not relevant	0	0.08	0	14.89	0	0	1
MCA (acid, I)	0.048	0.0123*	0.155*	0.12	0.07	2.02	1	0	1
MCA (salt, I)	0.112	0.0123*	0.155*	0.0069	0.48	$\infty$	1	0	1

\*Cf. Table 1.



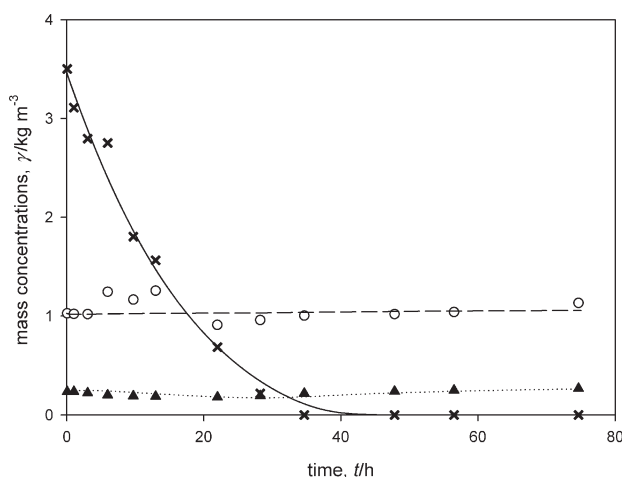


Fig. 7 – Comparison of experimental data for concentrations (squares) and modeling results (lines) for pulse of DBM. (x) – DBM,  $\text{kg m}^{-3}$ ; (o) – chloride,  $\text{kg m}^{-3}$ ; (▲) – biomass, (OD).

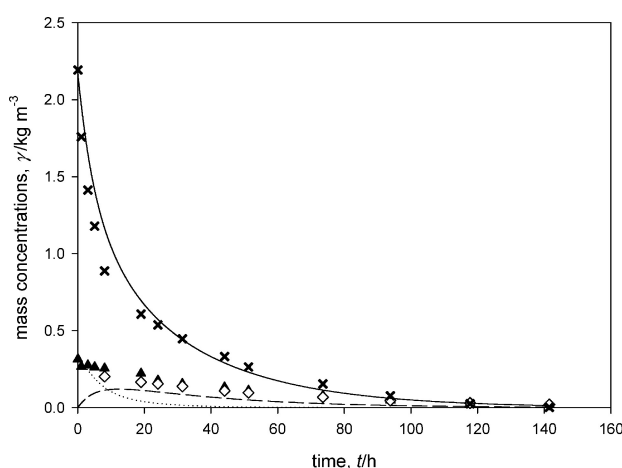


Fig. 8 – Comparison of experimental data for concentrations and modeling results (cf. the Appendix) for pulse of EDB. (x) – EDB,  $\text{kg m}^{-3}$ ; (◇) – bromide,  $\text{kg m}^{-3}$ ; (▲) – biomass, (OD).

In case of MCA (acid) the results of modeling lead to the conclusion that the dehalogenation rate of the substrate was much lower than when MCA was previously neutralized. Slow growth on MCA-acid occurred ( $\mu_{\max, I} = 0.048 \text{ h}^{-1}$ ). The value of the estimated toxicity coefficient  $\alpha$  was relatively high ( $\alpha = 11.3 \text{ M}^{-1} \text{ h}^{-1}$ ) probably because of the sudden drop of pH after the pulse. There are similar data in the literature for exponential cell death due to sudden pH change, cf. Kim *et al.*<sup>14</sup> The values for  $K_i$ , and  $\mu_{\max, I}$  (Table 3) estimated for the case of *monochloroacetic acid* correspond to relatively strong toxicity, slow microbial growth and slight utilization as a secondary carbon source. MCA could slow down growth on DCA since it is an intermediate in its metabolism.<sup>3</sup>

The variables estimated for MFA showed considerable toxic effect ( $\alpha = 6.23 \text{ M}^{-1} \text{ h}^{-1}$ ) with slight inhibition on growth on the main substrate. It does not serve as a secondary carbon source for additional microbial growth.

## Conclusions

The presented mathematical model was used for quantitative description of the combined effects of xenobiotic compounds added to CSTB: toxicity resulting in cell death and biomass wash-out (DBE, MCA, MFA), inhibition on the microbial growth on the main substrate (DBM, EDB, MFA MCA-acid) and/or additional growth due to use of the pulsed compound as an additional carbon source (DCM, DBM, MCA). In most cases the model curves fitted well to the experimental profiles proving that the model was sufficiently flexible to describe relatively complex biochemical behavior of the bioreactor.

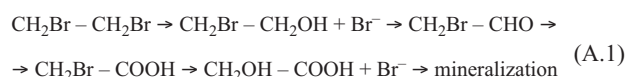
The numerical values for the model constants are useful for drawing conclusions about the nature of the effects that the added compounds exert on the strain: toxicity, leading to cell death; inhibition, leading to growth retardation and possibly consumption as a secondary carbon source and secondary conversion.

## ACKNOWLEDGEMENT

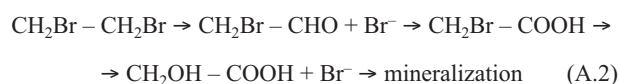
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## APPENDIX

The toxic effect of the intermediate products of DBE degradation on the overall biodegradation process could be demonstrated by the proposed mathematical model adapted for this case. One can consider a metabolic pathway similar to that for DCA, namely:



For the sake of brevity and due to the lack of experimental data for 2-bromoethanol and bromoacetaldehyde we shall consider the first two consecutive steps of this metabolic pathway as one, i.e.



Then the model equations will be presented as follows.

$$\begin{aligned}\frac{d\gamma_X}{dt} &= (\mu_S + \mu_I) \cdot \gamma_X - \alpha \cdot \gamma_B \cdot \gamma_X - D \cdot \gamma_X \\ \frac{d\gamma_S}{dt} &= D \cdot (\gamma_{S,inlet} - \gamma_S) - \frac{\mu_S}{Y_{X/S}} \gamma_X - k_L a \left( \gamma_S - \frac{\gamma_{S,g}}{H_S} \right) \\ \frac{d\gamma_{S,g}}{dt} &= k_L a \cdot \left( \gamma_S - \frac{\gamma_{S,g}}{H_S} \right) \frac{V_L}{V_g} - D_g \gamma_{S,g} \\ \frac{d\gamma_{I,g}}{dt} &= j \cdot k_L a \left( \gamma_I - \frac{\gamma_{I,g}}{H_I} \right) - j \cdot D_g \gamma_{I,g}\end{aligned}\quad (A.3)$$

$$\frac{d\gamma_P}{dt} = -D \cdot \gamma_P + 2 \frac{M_{Cl}}{M_S} \cdot \frac{\mu_S}{Y_{X/S}} \cdot \gamma_X + i \frac{M_{Cl}}{M_I} \gamma_X \left( \frac{\mu_I}{Y_{X/I}} + k \cdot \beta \cdot \gamma_I \right)$$

with the additional balance equations for DBE, BAA, MBA and bromide:

$$\begin{aligned}\frac{d\gamma_I}{dt} &= -D \cdot \gamma_I - \frac{\mu_I}{Y_{X/I}} \gamma_X - k \cdot \beta \cdot \gamma_X \cdot \gamma_I - j \cdot k_L a \left( \gamma_I - \frac{\gamma_{I,g}}{H_I} \right) - \lambda \gamma_X \gamma_I \\ \frac{d\gamma_B}{dt} &= \lambda \frac{M_{BAA}}{M_{DBE}} \gamma_X \gamma_I - D \cdot \gamma_B - \delta \cdot \gamma_X \gamma_B \\ \frac{d\gamma_{MBA}}{dt} &= \delta \frac{M_{MBA}}{M_{BAA}} \gamma_X \gamma_B - D \cdot \gamma_{MBA} - \kappa \cdot \gamma_X \gamma_{MBA} \\ \frac{d\gamma_{P_I}}{dt} &= \kappa \frac{M_{Br}}{M_{MBA}} \gamma_X \gamma_B - D \cdot \gamma_{P_I} + \lambda \frac{M_{Br}}{M_{DBE}} \gamma_X \gamma_I.\end{aligned}\quad (A.4)$$

Note that in the equations for microbial growth the bromoacetaldehyde concentration is inserted as a toxic substance. The initial conditions are:

$$t = 0, \quad \gamma_I = \gamma_{I,0}, \quad \gamma_B = \gamma_{MBA} = \gamma_{P_I} = 0. \quad (A.5)$$

The parameter evaluation procedure was identical to the one, described in Section "Modeling and discussion". The variables  $\alpha$ ,  $\lambda$ ,  $\delta$  and  $\kappa$  were evaluated minimizing the target function

$$\begin{aligned}\text{Sum} = \sum_i & \left[ (\gamma_{MBA,i}^{\text{calc}} - \gamma_{MBA,i}^{\text{exp}})^2 + (\gamma_{P,i}^{\text{calc}} - \gamma_{P,i}^{\text{exp}})^2 + \right. \\ & \left. + (\gamma_{I,i}^{\text{calc}} - \gamma_{I,i}^{\text{exp}})^2 + (\gamma_{X,tot,i}^{\text{calc}} - \gamma_{X,tot,i}^{\text{exp}})^2 \right]\end{aligned}\quad (A.6)$$

taking the other variables from Tables 1–3. The values of the obtained parameters are given in Table A.1. The results of this modeling, compared with the experimental data are shown in Fig. 8. The very good coincidence is evident.

Table A.1 – Toxicity parameter for BAA and rate constants in eqs. (A.4) evaluated from the experimental data for DBE pulse

$\alpha$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$	$\lambda$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$	$\delta$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$	$\kappa$ $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$
2.85	0.288	3.65	0.083

The review of the obtained quantitative results shows that the release of the first bromide ion goes faster ( $\lambda = 0.288 \text{ m}^3 \text{ kg}^{-1} \text{ h}^{-1}$ ) than the second one ( $\kappa = 0.083 \text{ m}^3 \text{ kg}^{-1} \text{ h}^{-1}$ ). The toxic effect of bromoacetaldehyde seems comparable to the one exerted by DBE only, cf. Table 3.

## Abbreviations

BAA – bromoacetaldehyde  
CSTB – continuous stirred tank bioreactor  
DBM – dibromomethane  
DBE – 1,2-dibromoethane  
DCA – 1,2-dichloroethane  
DCM – dichloromethane  
MBA – monobromoacetic acid  
MCA – monochloroacetate and monochloroacetic acid  
MFA – monofluoroacetate

## List of symbols

$D$  – dilution rate for the liquid phase (reciprocal to the hydraulic retention time),  $\text{h}^{-1}$   
 $D_g$  – dilution rate for the gaseous phase,  $\text{h}^{-1}$   
 $H$  – partition coefficients, i.e. Henry constants, (–)  
 $i$  – factor for the yield of chloride ions, equal to 0, 1 or 2  
 $j$  – factor for the substance volatility;  $j = 0$ , non-volatile, 1 – volatile  
 $k$  – factor for substrate degradation if not associated ( $k = 0$ ) or associated with cell growth ( $k = 1$ )  
 $K_i$  – inhibition constant,  $\text{kg m}^{-3}$   
 $k_L a$  – volumetric mass transfer coefficient,  $\text{h}^{-1}$   
 $K_S$  – saturation constant in Monod equation,  $\text{kg m}^{-3}$   
 $M$  – molar mass for certain compound, chloride and bromide as well  
 $Q$  – flow rate,  $\text{m}^3 \text{ h}^{-1}$   
 $r$  – rate of microbial growth,  $\text{kg m}^{-3} \text{ h}^{-1}$   
 $t$  – time, h  
 $V$  – volume of a given phase,  $\text{m}^3$   
 $Y$  – yield coefficients for cell growth,  $\text{kg}_{\text{cells}} \text{ kg}_{\text{substrate}}^{-1}$

## Greek symbols

$\alpha$  – toxicity parameter,  $(\text{kg m}^{-3})^{-1} \text{ h}^{-1}$  or  $\text{M}^{-1} \text{ h}^{-1}$   
 $\beta$  – first-order rate constant for degradation of secondary substrate,  $(\text{kg m}^{-3})^{-1} \text{ h}^{-1}$   
 $\gamma_B$  – concentration of bromoacetaldehyde,  $\text{kg m}^{-3}$   
 $\gamma_I$  – concentration of tested compound,  $\text{kg m}^{-3}$   
 $\gamma_{MBA}$  – concentration of monobromoacetic acid,  $\text{kg m}^{-3}$   
 $\gamma_P$  – product (chloride) concentration,  $\text{kg m}^{-3}$   
 $\gamma_{P_I}$  – bromide concentration,  $\text{kg m}^{-3}$   
 $\gamma_S$  – substrate (1,2-dichloroethane) concentration,  $\text{kg m}^{-3}$   
 $\gamma_{Sm}$  – inhibitory threshold concentration of substrate,  $1.08 \text{ kg m}^{-3}$   
 $\gamma_X$  – active biomass concentration,  $\text{kg m}^{-3}$   
 $\delta$  – rate constant of monobromoacetic acid formation,  $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$   
 $\kappa$  – rate constant of monobromoacetic acid degradation,  $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$   
 $\lambda$  – rate constant of bromoacetaldehyde formation from DBE,  $\text{m}^3 \text{ kg}^{-1} \text{ h}^{-1}$   
 $\mu$  – specific growth rate,  $\text{h}^{-1}$

## Subscripts

- g – refers to the gaseous phase
- I – refers to the secondary substrate (possible inhibitor and/or toxin)
- L – refers to the liquid phase
- max – denotes maximum value, i.e. maximum specific growth rate
- S – refers to the main substrate, i.e. DCA
- tot – related to the total biomass (alive and dead) read by the optical density
- 0 – denotes initial values

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